

Antinatriuretic Effect of Intramuscular Cadmium in Rats¹ (35466)

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Although the element cadmium is strikingly concentrated in the mammalian kidney (1) where it is bound to a particular and unusual protein, metallothionein (2), its physiological effects are unknown. Under appropriate circumstances relatively small doses have been reported to induce acute renal retention of sodium (3), both acute (4, 5) and chronic hypertension (6), and chronic proteinuria with slight impairment of renal function (7).

The present studies were designed to investigate the chronic renal effects of cadmium on electrolyte excretion. Small doses of cadmium, but doses which have been reported to be hypertensive (8), were used. The experimental animals were disturbed minimally during the course of the study: blood pressures were determined and blood was obtained only at the conclusion of the experiment. Rats receiving 4 injections of im (intramuscular) cadmium within 4 months were followed a total of 1 year; during the first 6 months, weekly weights were measured and urine collections were made. Total urinary sodium was diminished 24 to 48 hr after the first injection of cadmium; this early antinatriuretic effect increased after each successive cadmium injection. Moreover, confirmatory evidence for an early antinatriuretic response in cadmium-treated animals was provided by a temporary excessive weight gain. After the first 2 injections of cadmium, the antinatriuretic effect did not persist; within 1 week following injection, it had been replaced by a rebound natriuresis. After the third and fourth injections, however, no rebound natri-

uresis was observed. Unlike urinary sodium, urine volume and other urinary constituents, including protein, potassium, cadmium, and zinc were similar for cadmium-treated and control rats at all times studied.

At the time of sacrifice following a year of observation, evidence for persistent hypertension was sought. Although the blood pressure was not higher in the cadmium-treated than in the control rats, the 2 groups differed in two respects. Specifically, there was decreased vascular reactivity to a small dose of norepinephrine in the cadmium-treated group, and the peripheral renin levels of these animals were somewhat higher.

Methods. A total of 64 normal female rats of the Wistar strain, weighing from 180 to 200 g, were randomly separated: 32 animals to be given cadmium and 32 controls; for the metabolic studies, both groups were further divided into subgroups comprising 4 animals each. Once a week for 24 weeks, each subgroup of 4 rats was placed in a metabolism cage for 24 hr, and urine was collected. A final similar collection was made during the 52nd week of observation. Except during collection periods, the animals were given Purina rat chow and deionized metal-free water *ad libitum*. No food was available during collection periods, but deionized metal-free water remained freely available. Twenty-four hr before the fourth, eighth, thirteenth and nineteenth such collections, 1 mg of cadmium/kg of body weight was injected im into the hind leg of each "cadmium-treated" rat. The total dose of cadmium approximated 1 mg/animal, given over a period of 15 weeks. Each dose of cadmium was dissolved in 0.1 ml of 0.9% aqueous sodium chloride. Control rats received the saline only. Each subgroup

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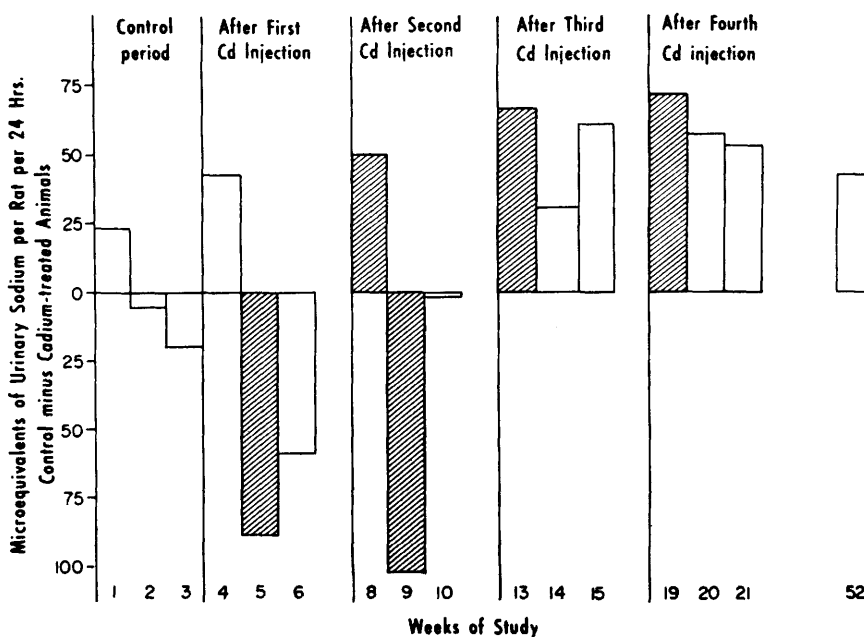


FIG. 1. Changes in sodium excretion following intramuscular cadmium: Each bar represents the average difference between the total urinary sodium excreted during a 24-hr period by each of 32 cadmium-treated and each of 32 control rats. The values for the cadmium-treated animals are subtracted from the values for the control animals so that bars extending above the zero line represent cadmium-induced retention of sodium. (shaded bars) indicate differences between the two groups of animals which were significant, with $p < 0.05$. Values are plotted for 15 of the 24 weekly collections, including the 3 weeks immediately before the first cadmium injection and the 3 weeks immediately after all four cadmium injections. Collections were always made on the same day of the week. The collections on the fourth, eighth, thirteenth, and nineteenth weeks began exactly 24 hr following a cadmium injection.

of 4 animals was weighed immediately before every urine collection. The volumes of the urine samples were measured; the concentrations of sodium and potassium in the samples were determined by flame photometry, of cadmium and zinc by atomic absorption, and of protein by the Lowry method.

During the 53rd week of observation and 1 week following the final urine collection, each animal was anesthetized with sodium pentothal and the blood pressure was directly measured from a needle in the femoral artery. Moreover, changes in blood pressure were measured following the intra-arterial injection of 10^{-9} g of angiotensin, following 10^{-8} g of norepinephrine and, finally following 2×10^{-5} g of cadmium, in that order. Each pressor agent was dissolved in 0.1 ml of 0.9% aqueous sodium chloride immediately prior to injection. The blood pressure was

recorded for 10 min after each intra-arterial injection or until it had returned to its control level, whichever was longer. Immediately after the blood pressure studies, the rats were sacrificed by rapid exsanguination and the kidneys and livers were removed. Renin levels were determined on the blood by previously-described methods (9) and the organs were weighed and assayed for cadmium by atomic absorption after ashing at low temperature under oxygen.

Results. Injection of cadmium intramuscularly was followed by an early decrease in the renal excretion of sodium. During the period from 24 to 48 hr after the 4 injections of 1 mg of cadmium/kg of body weight, injected animals excreted an average of 42, 50, 65, and 68 fewer microequivalents of sodium, respectively, in their urine than did control animals (Fig. 1). The last three of these

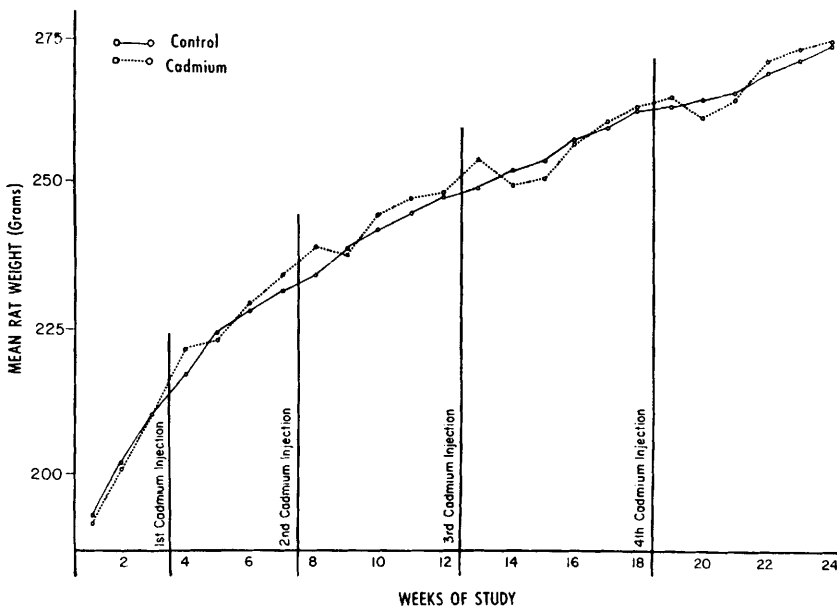


FIG. 2. Influence of intramuscular cadmium on body weight: The average weights of 32 cadmium-treated and 32 control rats, measured weekly for 24 weeks, are plotted. The weights were measured immediately before a 24-hr urine collection was begun. Therefore, rats were weighed 24 hr following each cadmium injection. After all four injections, the 24-hr postcadmium weight was temporarily excessive relative to the weight of the control animals, as might be expected if sodium retention had occurred.

differences were significant, with $p < 0.05$. A week after both of the first two cadmium injections, marked rebound natriuresis was significant ($p < 0.05$); no such rebound natriuresis was present after either the third or the fourth injection (Fig. 1). The early sodium retention following cadmium administration was reflected by a temporary increase in the weight of the cadmium-treated rats relative to the control rats during the 24 hr following each injection (Fig. 2). Aside from these temporary fluctuations, the weight gains of the 2 groups were very similar, thus failing to suggest any general deleterious effects of the cadmium. Sodium excretion was not significantly different for the 2 groups of animals during the 52nd week of observation, *i.e.*, 34 weeks following the last injection of cadmium (Fig. 1).

Urine volume showed an insignificant, but perhaps suggestive, relationship to cadmium injection, being low the first week after the second, third, and fourth injections and remaining low the second week after the third and fourth injections. Urinary potassium and

protein fluctuated little and were the same for cadmium-treated and control rats (Table I). No increase in urinary cadmium was detected after cadmium administration, and urinary cadmium and zinc levels were essentially the same in the cadmium-treated and control groups, averaging $6.8 \mu\text{g}$ of zinc and $0.25 \mu\text{g}$ of cadmium/day/rat.

One week after the final urine collection, *i.e.*, during the 53rd week of the experiment, differences in blood pressure, in vascular responsiveness and in peripheral renin activity were sought (Table II). The average blood pressure of the 32 cadmium-treated and 32 control rats was remarkably similar. There was an apparently significant decrease in the average response of the systolic pressure of cadmium-treated animals following 10^{-8} g of *l*-norepinephrine ($p < 0.05$). Although the average increase in both systolic and diastolic pressures following both angiotensin and cadmium were somewhat smaller for cadmium-treated than for control rats, none of these four differences between the 2 groups was significant. For the cadmium-treated rats, the

TABLE I. Comparison of Urinary Composition and Its Variability in "Cadmium-Treated" and "Control" Rats.^a

		Vol	Protein	Potassium	Sodium
Control	Min	2.31 ml/day	1.54 mg/day	418 μ Eq/24 hr	272 μ Eq/24 hr
	Max/min	1.42	1.28	1.40	1.41
Cadmium	Min	1.70 ml/day	1.48 mg/day	431 μ Eq/24 hr	210 μ Eq/24 hr
	Max/min	1.77	1.33	1.46	2.16

^a For each of 24 weeks, 24-hr collections were made for each of 8 groups of control and 8 groups of cadmium-treated rats. The 8 cadmium values and the 8 control values for each week were averaged. The smallest of these averages is cited above as "Min" and the ratio of the largest of these averages to this minimum is cited as "Max/min." Note that the values given here are for groups of 4 animals. Note also that for the control animals the variability of the four parameters (as indicated by "Max/min") ranges only from 1.28 to 1.42, whereas it is 2.16 for urinary sodium concentration of cadmium-treated rats.

level of peripheral renin averaged 1101 units; whereas, for the control rats, it averaged 839 units, the units being nanograms of angiotensin II per 100 ml of plasma following incubation for 1 hr at 37° and pH 5.7 (9). The large variability from animal to animal prevented the difference from being significant. As expected, there was considerable cadmium in the kidneys and livers of the cadmium-treated animals, the average values being 6.0 and 3.5 mg/g of tissue ash, respectively. For the kidney, the cadmium concentration was 81 and for the liver 15 times as high for the cadmium-treated as for the control animals.

Discussion. In man, cadmium is uniquely sequestered in the kidney: no other metal has a renal concentration which averages more than 10 times the concentration in the liver or other major organs (10). In view of

its predilection for kidney, cadmium might well be expected to have some persistent effect on renal function, and indeed it does. Chronic renal effects have been reported in rabbits by Axelsson and Piscator (7) who demonstrated that 12 to 20 mg of cadmium/kg of body weight, given in divided doses by subcutaneous injections over a period of 11 to 17 weeks, resulted in proteinuria, slight reduction in creatinine clearance, impaired reabsorption of glucose, and a reduction in alkaline phosphatase activity of renal cortex. In a subsequent communication involving the same animals, Axelsson *et al.* (11) reported anatomic renal changes consisting of cytoplasmic and nuclear degeneration in the epithelium of the proximal tubules.

The antinatriuretic effect described here followed each of 4 doses of parenteral cadmium similar to those known to induce chronic

TABLE II. Average Blood Pressure, Responsiveness to Pressor Agents, and Peripheral Blood Renin of "Control" and "Cadmium-Treated" Rats 6 Months Following the Last Cadmium Exposure.^a

	Av blood pressure (mm Hg)	Av increase in blood pressure (mm Hg) following			Av level of renin \pm SD
		Angiotensin (1×10^{-9} g)	Norepinephrine (1×10^{-8} g)	Cadmium (2×10^{-5} g)	
Control	146/104	8.5/5.8	8.3/6.1	9.6/6.4	839 \pm 419
Cadmium	142/103	7.8/5.7	5.3/5.2	8.8/4.4	1101 \pm 778

^a The values represent averages for the 32 cadmium and 32 control rats. Although the response to pressor agents was always less in the cadmium animals, the only statistical difference was in systolic pressure following norepinephrine ($p < 0.05$).

hypertension (8). This antinatriuretic effect, which became marked and more persistent with each exposure, to 1 mg of im cadmium/kg of body weight may represent the same phenomenon as the acute unilateral cadmium-induced increase in sodium resorption observed by Vander following infusion of 0.025 mg of cadmium/kg into 1 renal artery of a dog (3). Vander simultaneously observed a decrease in water excretion as well as a small and inconsistent decrease in potassium excretion. There was also a mild transient fall in glomerular filtration rate and renal plasma flow followed by temporary overcompensation. In subsequent stop-flow experiments on dogs undergoing mannitol diuresis, he localized the major site of action of intravenously infused cadmium to the proximal tubules, although some distal effect could not be excluded (12).

In our experiments, the pressor response to norepinephrine was decreased, a finding reminiscent of prior reports of cadmium altering vascular responsiveness, both *in vitro* and *in vivo*. Although no direct effect was noted, a spirally cut strip of rabbit aorta exposed to 10^{-4} M cadmium ion had a decreased response to epinephrine but an unchanged or increased response to angiotensin (13). After bathing aortic strips from normal rabbits in a wide range of cadmium concentrations for 0.5 to 4 hr, Thind and co-workers (14) found decreased responses to both norepinephrine and angiotensin. The same investigators found the aortic strips from rabbits made hypertensive with 2 mg of parenteral cadmium/kg of body weight had decreased responses to angiotensin, and that incubation with from 1 to 100×10^{-5} M cadmium ion further diminished responsiveness to angiotensin without affecting responses to norepinephrine (15). Youkilis *et al.* (16) found that 3 mg of cadmium/kg of body weight produced significant *in vivo* dilatation of venules and arterioles of innervated bat wings. Finally, Scott and Haddy found total peripheral resistance decreased in canine limbs during infusion of 3 to 24×10^{-4} M cadmium ion (17).

Summary. During the second day after an im injection of 1 mg of cadmium/kg of body weight, normal 200 g rats averaged 42 μ Eq less urinary sodium than control animals. This antinatriuretic effect increased with suc-

cessive doses of cadmium, reaching an average of 68 μ Eq after the fourth such dose. There was a corresponding increase in weight on the day after each cadmium injection. Rebound natriuresis was observed 1 and 2 weeks after the first dose of cadmium; it was less evident after the second dose and was completely absent after the third and fourth doses. Cadmium produced no corresponding change in urinary volume, protein, potassium, cadmium, or zinc. Six months after the fourth cadmium injection, there was no hypertension but there was a suggestive, if insignificant, increase in peripheral renin as well as a decrease in responsiveness to norepinephrine but not to either angiotensin or cadmium.

1. Tipton, I. H., Foland, W. D., Bobt, F. C., and McCorkle, W. C., *Phys. Rev.* **91**, (1953).

2. Kagi, J. H. R., and Vallee, B. L., *J. Biol. Chem.* **235**, 3460 (1960).

3. Vander, A. J., *Amer. J. Physiol.* **203**, 1 (1962).

4. Yunice, A., and Perry, H. M., Jr., *J. Lab. Clin. Med.* **58**, 975 (1961).

5. Perry, H. M., Jr., and Yunice, A., *Proc. Soc. Exp. Biol. Med.* **120**, 805 (1965).

6. Schroeder, H. A., and Vinton, W. H., Jr., *Amer. J. Physiol.* **202**, 515 (1962).

7. Axelsson, B., and Piscator, M., *Arch. Environ. Health* **12**, 374 (1966).

8. Schroeder, H. A., Kroll, S. S., Little, J. W., Livingston, P. O., and Myers, M. A. G., *Arch. Environ. Health* **13**, 788 (1966).

9. Bourgoignie, J. J., Catanzaro, F. J., and Perry, H. M., Jr., *Circulation* **37**, 27 (1968).

10. Perry, H. M., Jr., Tipton, I. H., Schroeder, H. A., Steiner, R. L., and Cook, M. J., *J. Chronic Dis.* **14**, 259 (1961).

11. Axelsson, B., Dahlgren, S. E., and Piscator, M., *Arch. Environ. Health* **17**, 24 (1968).

12. Vander, A. J., *Amer. J. Physiol.* **203** (6), 1005 (1962).

13. Perry, H. M., Jr., Schoepfle, E. S., and Bourgoignie, J. J., *Proc. Soc. Exp. Biol. Med.* **124**, 485 (1967).

14. Thind, G. S., Stephan, K. F., and Blakemore, W. S., *Clin. Res.* **16**, 519 (1968).

15. Thind, G. S., Stephan, K. F., and Blakemore, W. S., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **28**, 329 (1969).

16. Youkilis, E. J., Mutz, R. C., Harris, P. D., Nicoll, P. A., and Farney, R. B., *Toxicol. Appl. Pharmacol.* **14**, 31 (1969).

17. Scott, J. B., and Haddy, F. J., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **22**, 179 (1963).

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